This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

THIS PAGE BLANK (USPTO)

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 354 992 A2

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 89112463.8

(51) Int. Cl.[4] A61K 37/02 , C07K 7/10

(22) Date of filing: 07.07.89

The title of the invention has been amended (Guidelines for Examination in the EPO, A-III, 7.3).

(30) Priority: 08.07.88 IL 87055

(43) Date of publication of application: 21.02.90 Bulletin 90/08

(84) Designated Contracting States: AT BE CH DE ES FR GB GR IT LI LU NL SE

(71) Applicant: YEDA RESEARCH & DEVELOPMENT COMPANY, LIMITED
P.O. Box 95
Rehovot 76100(IL)

(72) Inventor: Gozes, Illana
30 Negba Street
Ness Ziona(IL)
Inventor: Fridkin, Matityahu
23 Miller Street
Rehovot(IL)

(74) Representative: Vossius & Partner
Siebertstrasse 4 P.O. Box 86 07 67
D-8000 München 86(DE)

(54) Topical pharmaceutical compositions comprising a vasoactive intestinal peptide.

(57) There are provided pharmaceutical compositions for topical transdermal application for the treatment of male impotence and compositions for the treatment of asthma and of hypertension. The active ingredients of such compositions are selected from vasoactive intestinal peptide (VIP), effective derivatives thereof and effective fragments thereof. In the compositions for the treatment of male impotence a transdermally effective conjugate of VIP, its derivatives or fragments coupled to a hydrophobic moiety, is used.

EP 0 354 992 A2

TOPICAL PHARMACEUTICAL COMPOSITIONS

The invention relates to pharmaceutical compositions for the treatment of male impotence.

Impotence, a condition that can bring much suffering to the life of the afflicted individual and those surrounding him, can be caused by psychological as well as organic problems. It is estimated that in the United States alone, 10 million men suffer from varying degrees of impotence. In general, impotence is a predominant syndrome affecting at least 10-15% of the male population. In men above 40 years of age, the occurrence of impotence is prevalent due to neuroendocrine failures associated with aging, and any man over the age of 40 years may experience occasional impotence. The mechanism of penile erection is a complicated one and requires the intactness of the endocrine system, the nervous system and the vascular system. Many times the suffering individual is hesitant to seek medical help and at the moment the remedies available to the physician are rather limited. Organic abnormalities can be treated by surgery and implantations. Erection was also obtained by injection of smooth muscle relaxants such as phenoxybenzamine. It is, of course, quite understandable that superior treatment is needed and the invention relates to an ointment to treat impotence.

It is the object of the invention to provide an improved pharmaceutical composition for the treatment of male impotence.This object is solved by the surprising finding that the transdermal application of vasoactive intestinal peptide (VIP) or derivatives or fragments thereof, coupled to a suitable hydrophobic moiety, enhances sexual activity.

Therefore, the subject matter of the invention is a transdermally effective pharmaceutical composition for topical application for the treatment of male impotence which comprises as active ingredient a physiologically effective quantity of a conjugate of vasoactive intestinal peptide (VIP), an active derivative or fragment thereof, coupled to a hydrophobic moiety.

The active ingredients are VIP, functional derivatives thereof, active fragments thereof, and modified peptides wherein one or more of the amino acids are substituted by others. The backbone of the compositions is a naturally occuring material, or derivative thereof.

The peptide of interest, Vasoactive Intestinal Peptide (VIP) fulfils several of the chemical criteria for a neurotransmitter in penile erection in the male: it is present in nerve fibers with nerve endings around cavernous smooth muscle and blood vessels and it is elevated during erection. Moreover, injection of exogenous VIP induces erection in man. It is important to add that in impotent men it was unequivocally shown that VIP quantities decrease locally in their penises. Since VIP was found to be a key neurotransmitter in erection formation, its local administration should help to relieve the dysfunction. Past studies used local VIP injection into the penis as the mode of drug administration. Using an animal model, we developed an ointment containing modified VIP which enhances mating behaviour in mammals, as evident from experiments with rats.

The behavioural model for studies of sexual behaviour was the castrated rat model. Castrated rats lose their sexual activity in a time dependent manner and we used this characteristic to develop a model for investigating the therapeutic activity of VIP.

The sequence of VIP, a 29-amino acid peptide, is as follows:

1 7

H-His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-

16

-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-

28

-Ser-Ile-Leu-Asn-NH_2

The peptide chain was assembled according to the solid-phase methodology (Merrifield (1963) J. Am. Chem. Soc. 85:2149). Partial sequences were also synthesized by this approach.

The following hydrophobic derivatives of VIP were prepared by attaching, as the last coupling step, a long chain aliphatic carboxylic acid to the N-terminus of the peptide chain. This was accomplished while the peptide was still attached to the polymeric support:

1. $CH_3(CH_2)_5CO$-VIP
2. $CH_3(CH_2)_{13}CO$-VIP
3. $CH_3(CH_2)_{13}CO$-VIP$_{7-28}$
4. $CH_3(CH_2)_{13}CO$-VIP$_{13-28}$

5. $CH(CH_2)_2-CH(CH_2)_4-CO$-VIP
   S ——— S

The following peptides were prepared by aminolysis of peptide-polymer conjungates with long-chain aliphatic amines; chain extension occurs at the C-terminus of the VIP:

1. VIP-CONH CH_2CH_3
2. VIP-CONH$(CH_2)_3CH_3$
3. VIP-CONH$(CH_2)_7CH_3$

All peptides were purified by HPLC and characterized by their amino acid analysis.

The following examples are to be construed in a non-limitative manner.

Examples

Synthesis of Stearyl-VIP

Synthesis was carried out via the solid-phase strategy. All amino acid derivatives were purchased from peptide Institute Inc. (Japan). It was performed either automatically using Peptide Synthesizer (Applied Biosystem) or manually. α -amino functional groups were protected by t-butyloxycarbonyl (t-Boc). Side-chain protecting groups were as follows: His, N^{im}-p-toluenesulfonyl; Lys, 2-chlorobenzyloxycarbonyl;p Tyr, 0-2.6-dichlorobenzyl; Arg, N^G-p-toluenesulfonyl; Asp, β -benzyl; Thr and Ser, O-benzyl. The first amino acid (Asn) was attached to benzhydryl amine resin (Chemalog) using N.N'-dicyclohexylcarbodiimide (DCC) as a coupling agent. All coupling stages were performed with a threefold excess of protected amino acid derivatives using DCC and 1-hydroxylbenzotriazole (HOBt) as coupling agents. All stages of coupling were monitored with ninhydrin. The stearic acid residue was added similarly to amino acid residues using DCC/HOBt. Final cleavage of the peptide from the polymeric support along with deprotection was achieved with anhydrous HF. The product was first purified by gel-filtration on a Sephadex G-25 column followed by HPLC on reversed phase C_{18}-column using a linear gradient obtained by mixing (A) 0.1% trifluoroacetic acid (TFA) in water and (B) 0.1% TFA in 70% acetonitril and 30% water. The product gave, after acidic hydrolysis, the expected ratio of its constituent amino acids.

Other peptides were similarly prepared.

It is clear that introduction of hydrophobic moieties into the peptide chain of VIP can also take place within the chain, e.g., at the amino side chains of Lys. The hydrophobic moieties can be aliphatic, linear saturated (as in the compounds prepared), linear unsaturated (containing double or triple bonds), branched, saturated and unsaturated, linear and branched containing heteroatoms, aromatic-aliphatic or aromatic, be introduced into either C, N terminus or within the peptide chain, or at two or more positions. All these modifications can be performed on fragments of VIP- as well, or on any other biologically active analog of VIP.

The animal model

All tested rats (Wistar derived from the departmental colony, Hormone Research Department, at the Weizmann Institute of Science), are sexually experienced male rats around 3 months of age (body weight ~ 250 g). At this age, the rats are castrated by removal of the testes and injected daily with testosterone for at

least 14 consecutive days.

All rats (males and females) that take part in the experiment are kept in a 12-hour light 12-hour dark cycle. Prior to testing, each male is put in a separate cage for at least 1 hour. A sexually receptive female is introduced to each male. Females were tested for their receptivity by vaginal smearings. The latencies of mounts and intromissions are recorded. The tests are terminated if the male does not mount within 15 minutes. Using castrated animals we achieved decreased sexual activity which is expressed by longer intervals between intromissions and less intromissions during the 15 min observation periods.

Initially, we tested a few VIP analogs by injection (intravenously 5-10 µg/animal) as a control we injected saline at identical volumes (5-10 µl).

Table 1 shows the results for stearyl-VIP injection at the upper left side of the chart we see that stearyl-VIP potentiates sexual activity in a comparable manner to VIP (right hand side of the table). This analog is lipophylic and hence may be a candidate for topical application.

As we have obtained positive results with stearyl-VIP by injection we have now developed a topical application method. This was achieved by mixing 20 µl of 1 µg/ml VIP-stearyl with 10 µl dimethyl sulfoxide (DMSO) and direct topical application on the sex organ. In Table II one can easily notice a remarkable sexual arousal after stearyl-VIP application compared with control rats subjected to DMSO. We therefore suggest that stearyl-VIP could be used as a potentiator of sexual activity in animals.

In Table III we compare the topical application of the carrier (DMSO) to stearic acid by itself, to VIP by itself and to stearyl-VIP. It is self-evident that stearyl-VIP is the most active analog. A shorter (8 amino acid) VIP analog which is related to the human immunodeficiency virus (peptide T) which did not show much activity upon topical application by itself is somewhat active in a conjugated form but not as VIP- stearyl.

(A)The effect of topical application of stearyl-VIP on the sexual behaviour of male rats are summarized in Table IV.

Preparation of Ointment

- Lanolin (1 g), water (3 g) DMSO (7 drops) and 10 mg of stearyl-VIP were manually homogenized in a mortar. An amount of paste, containing 30-50 µg peptide was applied onto the rat's penis and sexual behaviour, following the methodology previously described, was monitored. As shown in the Table, the peptide showed about 25% decrease in intromission latency and two-fold increase in ejaculations in 10 animals.

(B) Using the same protocol as in (A) an ointment of VIP-oleyl was prepared and tested. It did show a certain effect in shortening of intromission latency (~10% and also increased, though much less than stearyl-VIP, number of ejaculations.

The effecitve dose for humans is between 1 and 5 µg.

We claim that VIP-stearyl can be used in topical application to improve mammalian sexual behaviour in sexually deficient males. Moreover, we claim that further modification using the same idea of a lypophylic derivative may even improve the material as VIP is known to be a bronchodilator as well as a vasodilator. Other therapeutical applications such as in asthma and blood pressure regulation are also claimed.

Legends to tables

Table I

Rats were castrated and injected daily for 15 consecutive days with testosterone at 4 µg/100 g body weight (BW). Tests were performed with stearyl-VIP or VIP at 10 µg/animal.

Table II

Rats were castrated and injected daily for 16 consecutive days with testosterone at 2 µg/100 g BW. Testing was performed by topical application of stearyl-VIP-20 µg/20 µl mixed with 10 µl DMSO and compared to topical application of 30 µl DMSO.

Table III

6 rats. 120 days old. sexually experienced. were tested. The rats were castrated 14 days earlier and were injected with 4 µg 100 g BW of testosterone daily. In the table we see an increase in number of intromissions and shortening of latency after topical application of HIV·PeP T. stearyl and stearyl-VIP. All 6 materials were tested by topical application. Also there is a clear increase in the number of ejaculations after applications of HIV Pep T stearyl and stearyl-VIP. Corresponding results are expected with human males.

Table IV

Summary of the effect of topical application of stearyl-VIP on the sexual behvious of male rats.

TABLE I

Rat	Saline		VIP stearyl		Rat	Saline		VIP	
	Obs.	Intromission latency (seconds)	Obs.	Intromission latency		Obs.	Intromission latency	Obs.	Intromission latency
G	10	71±14:E	7(1M)	28± 7:E	A	9	41±2	26	30± 5:E
			4	50±17:E	A_1	9	54±4:E	15	27± 7:E
G_1	11(1M)	54± 9	12(1M)	33± 6:E	A_2	6	38±2	19	22± 2:E
G_2	23(8M)	30± 4	20(7M)	37± 6:E	B	9	59±6	10	56±10:E
F_1	10(3M)	78±20:E	14	56± 8:E					
F_3	2	101±21:E	6	45± 7:E	B_2	4	50±5	13	26±6:E
F	9	38± 7:E	7(4M)	17± 3:E					
	3	13± 6							

Obs. = Number of intromissions per 15 minute test period.
E = Ejaculations
M = Mountings without intromission

TABLE II

TOPICAL APPLICATION				
Rat	DMSO		Stearyl-VIP	
	Obs.	Intromission latency	Obs.	Intromission latency
A_1	11	48±12	21	23±4
A_2	8	79±30	14	29±4
A_3	-	-	14	32±8
B	21	31±5:E	10	16±3:E
			3	38±10
B_2	-	-	11(3M)	18±7
B_3	12	54±12	25	24±4

Please note that rats B_2 and A_3 were sexually active only after Stearyl-VIP application.

TABLE III

Rat	DMSO		HIV-PEPT-Stearyl		Stearic acid		Stearyl VIP		VIP	
	No. Ob.	Int. Latency	No. Ob.	Int. Latency	No. Ob.	Int. Latency	No. Ob.	Int. Latency	No. Ob.	Int. Latency
A	-	-	-	-	-	-	6	66±15	-	-
B	24	29±6E	21	23±3E	19	26±1E	28	18±3E	21	22±6
C	11	67±3E	16	28±2E	21	18±3E	20	18±2E	15	20±1
A_1	-	-	-	-	-	-	-	-	-	-
B_1	6	104±10	12	46±2	19	36±2E	49	12±3E	10	59±3
C_1	15	45±2	28	29±4E	-	-	31	26±1E	-	-

TABLE IV: The affect of stearyl-VIP on sexual behaviour of male rats

RATS	No treatment						control ointment						stearyl-VIP ointment					
	Exp. 1		Exp. 2		Exp. 3		Exp. 1		Exp. 2		Exp. 3		Exp. 1		Exp. 2		Exp. 3	
	Obs.	I.L.	Obs.	I.L.	Obs.	I.L.	Obs.	I.L.	Obs.	I.L.	Obs.	I.L.	Obs.	I.L.	Obs.	I.L.	Obs.	I.L.
1	9	40±1	10	39±5	13	30±7:E	12	32±3:E	13	30±3	11	44±3	12	36±3:E	13	30±3:E	11	40±4:E
2	15	25±2	11	39±9	12	36±5:E	12	30±1	11	36±5:E	10	47±2	11	32±5	11	40±3:E	12	36±5:E
3	10	40±5	9	45±5	12	37±3:E	13	26±4:E	14	24±2	12	41±5	12	29±4	12	39±4:E	11	39±3:E
4	11	37±5	9	41±3	9	42±5	10	40±5	11	39±6	10	46±3	11	28±5:E	10	37±5:E	11	23±4:E
5	13	30±2	10	39±5	10	40±7	11	36±3	12	37±2	11	40±2	9	46±4:E	11	46±2:E	13	20±5:E
6	11	36±3:E	14	31±7:E	13	32±6:E	13	33±2	14	28±3:E	12	38±4	11	44±2:E	10	50±3:E	11	40±1:E
7	17	20±6:E	13	30±5:E	15	27±3:E	14	29±1	12	36±5	15	22±5	12	39±5:E	11	41±3:E	14	22±7:E
8	9	40±7	10	41±6	9	42±3	11	39±3	12	32±3	16	16±4	13	39±1:E	12	39±3:E	10	40±3:E
9	8	42±3	11	38±5	12	37±4:E	13	29±6:E	12	36±2:E	12	32±3:E	10	48±4:E	11	46±4:E	9	40±7:E
10	14	27±5:E	13	33±3	14	28±3:E	13	31±1	14	26±2	14	22±5	12	26±3:E	12	27±4:E	13	20±2:E

I.L. = Intermission Latency (sec)

E = Ejaculations

EP 0 354 992 A2

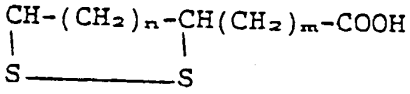
Claims

1. A transdermally effective pharmaceutical composition for topical application for the treatment of male impotence which comprises as active ingredient a physiologically effective quantity of a conjugate of vasoactive intestinal peptide (VIP), an active derivative or fragment thereof, coupled to a hydrophobic moiety.

2. A composition according to claim 1, where the hydrophobic moiety is of the formula CH_3-$(CH)_n$-CO-, where n is an integer of from 1 to 12.

3. A composition according to claim 1, where the VIP or its derivative or fragment is coupled to an entity of the formula -CO-NH-$(CH_2)_m$-CH_3, where m is an integer from 1 to 12.

4. A composition according to claim 1, where the VIP, its derivative or fragment is coupled to a moiety of the formula:



Where n is 2 to 6, and m is 2 to 12.

5. A composition according to any of claims 1 to 4, where the hydrophobic moiety is coupled to the VIP moiety via a side chain of one or more of the lysin groups, or via a hydroxy group of serine or threonine, through the side chain of aspergine or glutamine or through the C-terminal amino acid.

6. A composition according to claim 1 or 5, where the hydrophobic moiety is an aliphatic, aromatic, araliphatic, linear unsaturated, heterocyclic or alicyclic one, attached to either of the terminals of the VIP or VIP derivative or fragment polypeptides.

7. A composition according to claims 1 to 6, where the fragment is the 7-28 or the 16-28 fragment of VIP.

8. A pharmaceutical composition for alleviating the symptoms, and for the treatment of asthma, containing as active ingredient a conjugate defined in claim 1.

9. A pharmaceutical composition for the regulation of blood pressure containing an effective quantity of a conjugate defined in claim 1.

10. The use of a conjugate of vasoactive intestinal peptide (VIP), an active derivative or fragment thereof, coupled to a hydrophobic moiety for the preparation of a transdermally effective pharmaceutical composition for topical application for the treatment of male impotence, for the treatment of asthma or for the regulation of blood pressure.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 354 992 A3**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **89112463.8**

(51) Int. Cl.5: **A61K 37/02**, C07K 7/10

(22) Date of filing: **07.07.89**

(30) Priority: **08.07.88 IL 87055**

(43) Date of publication of application:
21.02.90 Bulletin 90/08

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(88) Date of deferred publication of the search report:
16.01.91 Bulletin 91/03

(71) Applicant: **YEDA RESEARCH AND DEVELOPMENT COMPANY LIMITED**
P.O. Box 95
Rehovot 76100(IL)

(72) Inventor: **Gozes, Illana**
30 Negba Street
Ness Ziona(IL)
Inventor: **Fridkin, Matityahu**
23 Miller Street
Rehovot(IL)

(74) Representative: **Vossius & Partnerner**
Siebertstrasse 4 P.O. Box 86 07 67
D-8000 München 86(DE)

(54) Topical pharmaceutical compositions comprising a vasoactive intestinal peptide.

(57) There are provided pharmaceutical compositions for topical transdermal application for the treatment of male impotence and compositions for the treatment of asthma and of hypertension. The active ingredients of such compositions are selected from vasoactive intestinal peptide (VIP), effective derivatives thereof and effective fragments thereof. In the compositions for the treatment of male impotence a transdermally effective conjugate of VIP, its derivatives or fragments coupled to a hydrophobic moiety, is used.

EP 0 354 992 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application number
EP 89112463.8

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	BIOLOGICAL ABSTRACT no. 35007263; A. SIDI et al.: "Patient acceptance of and satisfaction with vasoactive intracavernous..." & J. Urol. 1988, vol. 139, no. 4, part 2, page 328A	1-7	A61K37/02 C07K7/10
X	BIOLOGICAL ABSTRACT no. 33042928; A. SIDI et al.: "Short and long term complication of vasoactive intracavernous..." & J. Urol. 1987, vol. 137, no. 4, part 2, page 203A	1-7	
X,P	EP-A-297068 (KABIGEN AB) * page 2, line 40 *	1-10	
A	EP-A-225639 (MEJI SEIKA) * page 2 *	1-10	TECHNICAL FIELDS SEARCHED (Int. Cl.4) A61K C07K
A	US-A-3879371 (S. SAID) * the whole document *	1-7	
Y	US-A-4065641 (D. BOLIN) * column 2, line 63 *	8-10	
A	* column 4 *	1-7	

.../2

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Berlin	16.10.1990	P. AVEDIKIAN

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO Form 1503. 03.82

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
Y	PATENT ABSTRACTS OF JAPAN vol. 6, no. 3 (C-86)(881), 9 January 1982; & JP-A-56128721 (EISAI) 08.10.1981 * the whole abstract *	8,10	
X	PATENT ABSTRACTS OF JAPAN vol. 10, no. 303 (C-378)(2359), 16 October 1986; & JP-A-61118399 (OTSUKA) 05.06.1986 * the whole abstract *	9,10	
X	EP-A-241926 (EISAI) * page 23, lines 15-18; claim 8 *	8,9,10	TECHNICAL FIELDS SEARCHED (Int. Cl.4)
A	EP-A-184309 (BEECHAM) * the whole document *	8,9,10	

European Patent Office

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid,

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

X LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,
namely:

1. claims: 1-7 VIP derivative and composition for treating impotency

2. claims: 8,10 Second therapeutic application: asthma

3. claims: 9,10 Third therapeutic application: blood pressure

☒ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid,

namely claims:

☐ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims,

namely claims: